

a change in the amount of free radical damage and, therefore, of the effectiveness or ineffectiveness of the treatment, the determination being made by:

(a) contacting each of the biological samples with an excess quantity of a metal ion salt, the metal ion being capable of binding to the N-terminus of unmodified human albumin, to form a mixture of bound metal ions and unbound metal ions;

(b) determining the amount of bound metal ions for each biological sample, the amount of bound metal ions providing a measure of the quantity of the modified albumin in the sample; and

(c) determining if there is a change in the amount of bound metal ions between the first and subsequent sample(s).

49. The method of Claim 48 wherein the compound is a free radical scavenger.

50. The method of Claim 49 wherein the free radical scavenger is selected from the group consisting of superoxide dismutase, catalase, glutathione peroxidase, ebselen, glutathione, cysteine, N-acetyl cysteine, penicillamine, allopurinol, oxypurinol, ascorbic acid,  $\alpha$ -tocopherol, water-soluble  $\alpha$ -tocopherol,  $\beta$ -carotene, fatty-acid binding protein, fenozan, probucol, cyanidanol-3, dimercaptopropanol, indapamide, emoxipine, and dimethyl sulfoxide.

51. The method of Claim 48 wherein the compound is a photosensitizing agent used in photodynamic therapy for the treatment of tumors.

52. The method of Claim 51 wherein the compound is porfimer sodium.

53. The method of Claim 48 wherein said sample is serum or plasma.

54. The method of Claim 48 wherein said sample is purified albumin.

55. The method of Claim 48 wherein said metal ion salt is a salt of a transition metal ion of Groups 1b-7b or 8 of the Periodic Table of the elements.

56. The method of Claim 48 wherein said metal ion salt is a salt of a metal selected from the group consisting of V, As, Co, Sb, Cr, Mo, Mn, Ba, Zn, Ni, Hg, Cd, Fe, Pb, Au and Ag.

57. The method of Claim 48 wherein said metal ion salt is a salt of cobalt.

58. The method of Claim 48 wherein step (b) is conducted using atomic absorption or atomic emission spectroscopy.

59. The method of Claim 48 wherein step (b) is conducted using an immunological assay.

60. The method of Claim 48 wherein said excess quantity of metal ion salt is a predetermined quantity, and the quantity of unbound metal ions is detected to determine the amount of bound metal ions.

61. The method of claim 60 wherein detection of the quantity of unbound metal ions is conducted using atomic absorption or atomic emission spectroscopy.

62. The method of Claim 60 wherein detection of the quantity of unbound metal ions is conducted using an immunological assay.

63. The method of Claim 60 further comprising the steps of:

adding a compound having the formula Asp-Ala-His-Lys-R, wherein R is a group capable of being detected when the compound is bound to the metal ion of said metal ion salt, to the mixture containing bound metal ions and unbound metal ions; and

detecting the quantity of R to detect the quantity of unbound metal ions.

64. The method of Claim 63 wherein the determination of the quantity of said compound which is complexed with said metal ion is conducted using atomic absorption or atomic emission spectroscopy.

65. The method of Claim 63 wherein the determination of the quantity of said compound which is complexed with said metal ion is conducted using an immunological assay.

66. The method of Claim 60 wherein:

the metal ion salt is a salt of a metal selected from the group consisting of V, As, Co, Sb, Cr, Mo, Mn, Ba, Zn, Ni, Hg, Cd, Fe, Pb, Au and Ag;

the mixture of bound metal ions and unbound metal ions is contacted with an aqueous color forming compound solution to form a colored solution, said compound being able to form color when bound to the metal ions; and

the color intensity of said colored solution is determined to determine the quantity of unbound metal ions to provide a measure of bound metal ions.

67. The method of Claim 66 wherein said color forming compound is ferrozine.

68. The method of Claim 66 wherein said metal ion salt is a salt of cobalt.

69. A method of ~~monitoring or assessing~~ treatment of a disease or condition with a compound that produces or ~~reduces~~ free radicals comprising:

obtaining a first purified albumin sample from a first biological sample from a patient suffering from the disease or condition;

treating the patient with the compound;

obtaining one or more additional purified albumin samples from the same type of biological sample from the same patient at one or more times after the treatment; and

determining if there is a change in the quantity of a modified albumin present in the first purified albumin sample as compared to the subsequent sample(s), the modified albumin having a reduced ability to bind metal ions at its N-terminus, the quantity of the modified albumin in each sample being indicative of the amount of free radical damage in that sample, and a change in the quantity of the modified albumin in the first sample as compared to the subsequent sample(s) being indicative of a change in the amount of free radical damage and, therefore, of the effectiveness or ineffectiveness of the treatment, the determination being made by:

detecting the amount of copper ions present in each of the purified albumin samples, the quantity of copper ions providing a measure of the quantity of the modified albumin present in the sample; and

determining if there is a change in the amount of copper ions between the first purified albumin sample and the subsequent sample(s).

70. The method of Claim 69 wherein the compound is a free radical scavenger.

71. The method of Claim 70 wherein the free radical scavenger is selected from the group consisting of superoxide dismutase, catalase, glutathione peroxidase, ebselen, glutathione, cysteine, N-acetyl cysteine, penicillamine, allopurinol, oxypurinol, ascorbic acid,  $\alpha$ -tocopherol, water-soluble  $\alpha$ -tocopherol,  $\beta$ -carotene, fatty-acid binding protein, fenozan, probucol, cyanidanol-3, dimercaptopropanol, indapamide, emoxipine, and dimethyl sulfoxide.

72. The method of Claim 69 wherein the compound is a photosensitizing agent used in photodynamic therapy for the treatment of tumors.

73. The method of Claim 72 wherein the compound is porfimer sodium.

74. The method of Claim 69 wherein said detecting step is conducted using an immunological assay.

75. The method of Claim 69 wherein said detecting step is conducted using an atomic absorption or atomic emission spectroscopy.

76. A method of monitoring or assessing treatment of a disease or condition in which free radicals play a role comprising:

obtaining a first biological sample containing albumin from a patient suffering from the disease or condition;

treating the patient;

obtaining one or more additional biological samples of the same type from the same patient at one or more times after the treatment; and

determining if there is a change in the quantity of a modified albumin present in the first sample as compared to the subsequent sample(s), the modified albumin having a reduced ability to bind metal ions at its N-terminus, the quantity of the modified albumin in each sample being indicative of the amount of free radical damage in that sample, and a change in the quantity of the modified albumin in the first sample as compared to the subsequent sample(s) being indicative of a change in the amount of free radical damage and, therefore, of the effectiveness or ineffectiveness of the treatment, the determination being made by:

(a) contacting each of the biological samples with an excess quantity of a metal ion salt, the metal ion being capable of binding to the N-terminus of unmodified human albumin, to form a mixture of bound metal ions and unbound metal ions;

(b) determining the amount of bound metal ions for each biological sample, the amount of bound metal ions providing a measure of the quantity of the modified albumin in the sample; and

(c) determining if there is a change in the amount of bound metal ions between the first and subsequent sample(s).

77. The method of Claim 76 wherein the treatment is selected from the group consisting of surgery, radiation therapy, chemotherapy, reperfusion of an ischemic tissue or organ, and administration of a free radical scavenger.

78. The method of Claim 76 wherein said sample is serum or plasma.

79. The method of Claim 76 wherein said sample is purified albumin.

80. The method of Claim 76 wherein said metal ion salt is a salt of a transition metal ion of Groups 1b-7b or 8 of the Periodic Table of the elements.

81. The method of Claim 76 wherein said metal ion salt is a salt of a metal selected from the group consisting of V, As, Co, Sb, Cr, Mo, Mn, Ba, Zn, Ni, Hg, Cd, Fe, Pb, Au and Ag.

82. The method of Claim 76 wherein said metal ion salt is a salt of cobalt.

83. The method of Claim 76 wherein step (b) is conducted using atomic absorption or atomic emission spectroscopy.

84. The method of Claim 76 wherein step (b) is conducted using an immunological assay.

85. The method of Claim 76 wherein said excess quantity of metal ion salt is a predetermined quantity, and the quantity of unbound metal ions is detected to determine the amount of bound metal ions.

86. The method of claim 85 wherein detection of the quantity of unbound metal ions is conducted using atomic absorption or atomic emission spectroscopy.

87. The method of Claim 85 wherein detection of the quantity of unbound metal ions is conducted using an immunological assay.

88. The method of Claim 85 further comprising the steps of:

adding a compound having the formula Asp-Ala-His-Lys-R, wherein R is a group capable of being detected when the compound is bound to the metal ion of said metal ion salt, to the mixture containing bound metal ions and unbound metal ions; and

detecting the quantity of R to detect the quantity of unbound metal ions.

89. The method of Claim 88 wherein the determination of the quantity of said compound which is complexed with said metal ion is conducted using atomic absorption or atomic emission spectroscopy.

90. The method of Claim 88 wherein the determination of the quantity of said compound which is complexed with said metal ion is conducted using an immunological assay.

91. The method of Claim 85 wherein:

the metal ion salt is a salt of a metal selected from the group consisting of V, As, Co, Sb, Cr, Mo, Mn, Ba, Zn, Ni, Hg, Cd, Fe, Pb, Au and Ag;

the mixture of bound metal ions and unbound metal ions is contacted with an aqueous color forming compound solution to form a colored solution, said compound being able to form color when bound to the metal ions; and

the color intensity of said colored solution is determined to determine the quantity of unbound metal ions to provide a measure of bound metal ions.

92. The method of Claim 91 wherein said color forming compound is ferrozine.

93. The method of Claim 91 wherein said metal ion salt is a salt of cobalt.

94. A method of monitoring or assessing treatment of a disease or condition in which free radicals play a role comprising:

obtaining a first purified albumin sample from a first biological sample from a patient suffering from the disease or condition;

treating the patient;

obtaining one or more additional purified albumin samples from the same type of biological sample from the same patient at one or more times after the treatment; and

determining if there is a change in the quantity of a modified albumin present in the first purified albumin sample as compared to the subsequent sample(s), the modified albumin having a reduced ability to bind metal ions at its N-terminus, the quantity of the modified albumin in each sample being indicative of the amount of free radical damage in that sample, and a change in the quantity of the modified albumin in the first sample as compared to the subsequent sample(s) being indicative of a change in the amount of free radical damage and, therefore, of the effectiveness or ineffectiveness of the treatment, the determination being made by:

detecting the amount of copper ions present in each of the purified albumin samples, the quantity of copper ions providing a measure of the quantity of the modified albumin present in the sample; and

determining if there is a change in the amount of copper ions between the first purified albumin sample and the subsequent sample(s).

95. The method of Claim 94 wherein the treatment is selected from the group consisting of surgery, radiation therapy, chemotherapy, reperfusion of an ischemic tissue or organ, and administration of a free radical scavenger.

96. The method of Claim 94 wherein said detecting step is conducted using an immunological assay.

97. The method of Claim 94 wherein said detecting step is conducted using an atomic absorption or atomic emission spectroscopy.

98. A method of monitoring or assessing a disease or condition in which free radicals play a role comprising:

obtaining a first biological sample containing albumin from a patient suffering from the disease or condition;

obtaining one or more additional biological samples of the same type from the same patient at one or more times after obtaining the first sample; and

determining if there is a change in the quantity of a modified albumin present in the first sample as compared to the subsequent sample(s), the modified albumin having a reduced ability to bind metal ions at its N-terminus, the quantity of the modified albumin in each sample being indicative of the amount of free radical damage in that sample, and a change in the quantity of the modified albumin in the first sample as compared to the subsequent sample(s) being indicative of a change in the amount of free radical damage and, therefore, of the status of the disease or condition, the determination being made by:

(a) contacting each of the biological samples with an excess quantity of a metal ion salt, the metal ion being capable of binding to the N-terminus of unmodified human albumin, to form a mixture of bound metal ions and unbound metal ions;

(b) determining the amount of bound metal ions for each biological sample, the amount of bound metal ions providing a measure of the quantity of the modified albumin in the sample; and

(c) determining if there is a change in the amount of bound metal ions between the first and subsequent sample(s).

99. A method of monitoring or assessing a disease or condition in which free radicals play a role comprising:

obtaining a first purified albumin sample from a first biological sample from a patient suffering from the disease or condition;

obtaining one or more additional purified albumin samples from the same type of biological sample from the same patient at one or more times after the first sample; and

determining if there is a change in the quantity of a modified albumin present in the first purified albumin sample as compared to the subsequent sample(s), the modified albumin having a reduced ability to bind metal ions at its N-terminus, the quantity of the modified albumin in each sample being indicative of the amount of free radical damage in that sample, and a change in the quantity of the modified albumin in the first sample as compared to the subsequent sample(s) being indicative of a change in the amount of free radical damage and, therefore, of the status of the disease or condition, the determination being made by:

detecting the amount of copper ions present in each of the purified albumin samples, the quantity of copper ions providing a measure of the quantity of the modified albumin present in the sample; and

determining if there is a change in the amount of copper ions between the first purified albumin sample and the subsequent sample(s).

100. A method of detecting or quantitating free radicals in an excised tissue or organ comprising:

obtaining a biological sample containing albumin from the tissue or organ; and

detecting or quantifying a modified albumin in the sample, the modified albumin having a reduced ability to bind metal ions at its N-terminus, the presence and quantity of the modified albumin being indicative of the presence and amount of free radical damage, the determination being made by:

(a) contacting the biological sample with an excess quantity of a metal ion salt, the metal ion being capable of binding to the N-terminus of unmodified human albumin, to form a mixture of bound metal ions and unbound metal ions; and

(b) determining the amount of bound metal ions, the amount of bound metal ions providing a measure of the quantity of the modified albumin in the sample.

101. A method of detecting or quantitating free radicals in an excised tissue or organ comprising:

obtaining a purified albumin sample from the tissue or organ; and



*As concurred*  
detecting or quantifying a modified albumin present in the purified albumin sample, the modified albumin having a reduced ability to bind metal ions at its N-terminus, the presence and quantity of the modified albumin in each sample being indicative of the presence and amount of free radical damage, the determination being made by:

detecting the amount of copper ions present in the purified albumin sample, the quantity of copper ions providing a measure of the quantity of the modified albumin present in the sample.--

---

#### REMARKS

After the above amendments, Claims 48-101 are pending. These amendments are being made to more fully claim certain aspects of the invention.

Support for the new claims may be found throughout the application, including the claims as originally filed. See particularly page 12, line 26 through page 15, line 14, and Examples 12, 16 and 17. It is submitted that the pending claims are in condition for allowance, and a speedy allowance of them is requested.

Respectfully submitted,

SHERIDAN ROSS P.C.

By: Wannell M. Crook

Wannell M. Crook  
Registration No. 31,071  
1560 Broadway, Suite 1200  
Denver, CO 80202-5141  
(303) 863-9700

Date: July 23, 2001